

1. (currently amended) A method for determining the level of resistance of HIV to an HIV RT inhibitor, comprising:

the following assay components or reagents

a) providing a reaction well with ~~entities that result from the interaction~~ the reaction products of substances comprising

at least one template for an HIV RT enzyme,

at least one primer,

at least one detectable dNTP substrate,

at least one HIV RT inhibitor,

at least one ribonucleotide chosen from ATP and GTP, or at least one

pyrophosphate;

b) performing an enzymatic kinetics assay that permits the measurement of multiple chain termination events by adding to the reaction well an HIV RT enzyme chosen from a wild-type RT enzyme, wherein said HIV RT enzyme incorporates the at least one detectable dNTP substrate or at least one HIV RT inhibitor into said template;

c) determining RT activity by measuring the amount of the detectable dNTP substrate incorporated into the template;

d) repeating steps b) and c) replacing the wild-type RT enzyme with a mutant RT enzyme; and

e) determining the level of resistance of HIV to the HIV RT inhibitor by comparing the RT activity of the wild-type RT enzyme with the RT activity of the mutant RT enzyme.

2. (original) The method of claim 1, wherein the template is bound to the reaction well and is chosen from poly-rA or a heteropolymer RNA or DNA.

3. (original) The method of claim 1, wherein the primer is chosen from oligo-dt or a primer that is complementary to the heteropolymer template.
4. (original) The method of claim 1, wherein the detectable dNTP substrate is chosen from a radioactive labeled dNTP.
5. (original) The method of claim 1, wherein the detectable dNTP substrate is capable of being detected by fluorescence, luminescence, or absorption spectrometry.
6. (original) The method of claim 1, wherein the detectable dNTP substrate binds to an optical tracer or a radioactive labeled tracer.
7. (original) The method of claim 6, wherein the optical tracer is capable of being detected by fluorescence, luminescence, or absorption spectrometry.
8. (original) The method of claim 6, wherein the detectable dNTP precursor is bromo-deoxyuridine-triphosphate.
9. (original) The method of claim 7, wherein the optical tracer is a monoclonal anti-BrdU antibody, conjugated to alkaline phosphatase.
10. (original) The method of claim 1, wherein the HIV RT inhibitor is chosen from AZT, 3TC, ddI, ddC, d4T, and abacavir.

Serial No. 09/599,877

11. (original) The method of claim 1, wherein the HIV RT inhibitor is chosen from a nucleoside or a nucleoside analog.
12. (original) The method of claim 11, wherein the HIV RT inhibitor is a triphosphate form of the HIV RT inhibitor.
13. (original) The method of claim 1, wherein the mutant RT enzyme contains mutations at codons 67, 69 and 70.
14. (currently amended) The method of claim 1, wherein the HIV-1 mutant RT enzyme contains an insertional mutation at nucleotide triplet encoding codon 69.

*amino acid insertion between
RT codons 69 and 70.*

Serial No. 09/599,877

15. (canceled)

16. (canceled)

17. (canceled)

18. (canceled)

19. (canceled)

20. (currently amended) A method for determining the effect of at least one mutation in an HIV RT enzyme on the resistance of HIV to an HIV RT inhibitor, comprising:

a) providing a reaction well with ~~entities that result from the interaction~~ the reaction products of substances comprising

at least one template for an HIV RT enzyme,

at least one primer,

at least one detectable dNTP substrate,

at least one HIV RT inhibitor, and

at least one ribonucleotide chosen from ATP or GTP_γ or at least one pyrophosphate;

b) performing an enzymatic kinetics assay that permits the measurement of multiple chain termination events by adding to the reaction well an HIV RT enzyme, wherein said HIV RT enzyme incorporates the at least one detectable dNTP substrate or the at least one HIV RT inhibitor into said template;

c) determining RT activity by measuring the amount of the detectable dNTP substrate incorporated into the template;

d) repeating steps a) through c) in a new reaction well wherein the HIV RT enzyme of step b) is chosen from at least one mutant RT enzyme;

e) comparing the RT activity in the different reaction wells; and

f) determining the effect of the at least one mutation on the resistance of HIV to an HIV RT inhibitor using step e).

21. (currently amended) A method for rapid screening the effects of mutations on HIV resistance to an HIV RT inhibitor, comprising:

a) providing an array of reaction wells, each reaction well with ~~entities that result from the interaction~~ the reaction products of substances comprising:

at least one template for an HIV RT enzyme,

at least one primer,

at least one detectable dNTP substrate,

at least one HIV RT inhibitor, and

at least one ribonucleotide chosen from ATP or GTP_γ or at least one pyrophosphate;

b) performing an enzymatic kinetics assay that permits the measurement of multiple chain termination events by adding to each reaction well a different HIV RT enzyme chosen from a wild-type RT enzyme or a mutant RT enzyme, wherein said HIV RT enzyme incorporates the at least one detectable dNTP substrate or the at least one HIV RT inhibitor into said template and wherein at least one wild-type RT enzyme is added to at least one reaction well;

c) determining RT activity in each reaction well by measuring the amount of the detectable dNTP substrate incorporated into the template; and

d) determining the effect of mutations on HIV resistance of the HIV RT inhibitor by comparing the RT activity of at least one wild-type RT enzyme with the RT activity of at least one mutant RT enzyme.